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**Strigolactones cross the kingdoms: plants, fungi and bacteria in the arbuscular mycorrhizal symbiosis**

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## Abstract

Strigolactones firstly evolved as regulators of simple developmental processes in very ancient plant lineages and then assumed new roles to sustain the increasing biological complexity of land plants. Their versatility is also witnessed by the fact that during the evolution they have been exploited, once released in the rhizosphere, as a communication system towards plant-interacting organisms even belonging to different kingdoms. Here we reviewed the impact of SLs on soil microbes giving attention in particular to arbuscular mycorrhizal fungi (AMF). SLs induce several responses in AMF, including spore germination, hyphal branching, mitochondrial metabolism, transcriptional reprogramming and production of chitin oligosaccharides which, in turn, stimulate early symbiotic responses in the host plant. In the specific case study of the AMF *Gigaspora margarita*, SLs are also perceived, directly or indirectly, by the well characterized population of endobacteria with an increase of bacterial divisions and the activation of specific transcriptional responses. SLs dynamic during AM root colonization was also surveyed. Although not essential for the establishment of this mutualistic association, SLs act as positive regulators as they are relevant to achieve a full extent of colonization. This possibly occurs through a complex cross-talk with other hormones such as auxin, abscisic acid and gibberellins.

**Key words:** arbuscular mycorrhizal fungi, endobacteria, fungi, hormones, mutants, root symbiosis, strigolactones

## Abbreviations

ABA: abscisic acid

AMF: arbuscular mycorrhizal fungi

BR: brassinosteroids

CK: cytokinines

CSP: common symbiotic pathway

GA: gibberellin

SLs: strigolactones

**Running title:** Strigolactones cross the kingdoms

32 **Highlight:**

33 Strigolactones are versatile plant molecules used not only as hormones but also as a  
34 communication system to regulate the AM symbiosis through the activation of multiple  
35 responses.

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## Introduction

Among plant-associated microbes, the widespread arbuscular mycorrhizal fungi (AMF) play a key role in nutrient cycling and plant health due to their ability to improve plant mineral nutrition and tolerance to biotic and abiotic stresses. These fungi belong to an ancient monophyletic group, the Glomeromycotina (Spatafora *et al.*, 2016). AMF are obligate biotrophs with coenocytic hyphae and multinucleated asexual spores, although recently hidden sexuality events were proposed to occur (Corradi and Brachmann, 2017). Since AMF establish interactions with more than 80% of land plants, including basal plants like bryophytes and crop plants (Bonfante and Genre, 2010), and may also host endobacteria in their cytoplasm (Bonfante and Desirò, 2017), the AM symbiosis is an excellent model to discuss the exchange of signaling molecules at the inter-kingdom and inter-domain level. Plants have to distinguish among the surrounding microbes the friends or the foes, while AMF have to identify the photosynthetic host which guarantees a flow of reduced carbon. Recent papers have demonstrated that host plants provide lipids to their fungal partners (Bravo *et al.*, 2017; Luginbuehl *et al.*, 2017; Jiang *et al.*, 2017; Keymer *et al.*, 2017) and not only sugars as claimed for many years. In turn, AMF transfer to the host plants mineral nutrients. These exchanges are thought to occur primarily in root cortical cells hosting highly branched fungal hyphae, called arbuscules, which are therefore considered key structures of a functional symbiosis (Gutjahr and Parniske, 2013).

While the existence of a conserved signaling transduction pathway, usually defined as the common symbiotic pathway (CSP) since shared by the AM and the rhizobia-legumes symbioses, has been the object of many investigations and summarized in excellent reviews (Oldroyd, 2013; Genre and Russo, 2016; Zipfel and Oldroyd 2017), plant and fungal molecules that trigger symbiotic responses in the corresponding AM partner are less well characterized. Bonfante and Genre (2015) have proposed the hypothesis that the molecules involved in inter-kingdom symbiotic signaling, such as strigolactones (SLs), cutin monomers, and chitin-related molecules, also have key roles in development, originally unrelated to symbiosis. Thus, the symbiotic role of these molecules relies on the co-evolved capacity of the AM partners to perceive them as symbiotic signals.

Not only chitin oligosaccharides, but also SLs well fit to this suggestion. SLs derive from carotenoid metabolism (Al Babili and Bouwmeester, 2015); they were first studied as root-exuded molecules that elicit the germination of parasitic plants (Cook *et al.*, 1966). More

recently, SLs were acknowledged as bioactive molecules that stimulate the branching and metabolism of pre-symbiotic hyphae in AMF (Akiyama *et al.*, 2005, Besserer *et al.*, 2006). Finally, SLs emerged as key plant hormones that control several aspects of plant biology and physiology such as the repression of shoot branching (Gomez-Roldàn *et al.*, 2008; Umehara *et al.*, 2008; Waters *et al.*, 2017), the regulation of root system architecture (Koltai *et al.*, 2011; Kapulnik and Koltai, 2014; Sun *et al.*, 2016), the formation of adventitious root and leaf senescence (Waters *et al.*, 2017). SLs production is conserved from Charales to Embryophytes (Delaux *et al.*, 2012). Their function in the rhizosphere seems to be a secondary feature relying on their active release from the roots into the soil (Kretzschmar *et al.*, 2012).

In conclusion, emerging data suggest that SLs function as conserved determinants of plant development that were recruited during the evolution of plant symbiotic and parasitic interactions (Waters *et al.*, 2017).

The aim of the review is to focus on the SLs when released into the rhizosphere: in detail, we will summarize the direct impact of SLs on soil microbes, which proliferate in this specific niche, giving attention to AM and pathogenic fungi. Since these microbes interact with plants, we also review current knowledge on SLs dynamic during plant-microbe interactions, in particular on how the plants regulate SLs synthesis during the colonization. Lastly, we will provide information obtained from the analyses of plant mutants defective in the biosynthesis or in the perception of SLs and highlight how the cross-talk with other hormones could contribute to the control of the extent of plant colonization.

### **Strigolactones: their impact on arbuscular mycorrhizal fungi**

Being released in the rhizosphere, SLs have potential effects on microbes which proliferate in the soil around the roots. Special attention has been given so far to the symbiotic microbes, AMF and rhizobia (Waters *et al.*, 2017), while only a few reports have investigated how saprotrophic or pathogenic fungi respond to SLs.

Akiyama and colleagues (2005; 2010) first described how SLs lead to a specific phenotype during the pre-symbiotic phase of AMF. They based their work also on the use of GR24, a synthetic SLs analog. It is worth to note that several studies on SLs have been carried out using GR24, normally used as a racemic solution of the two enantiomers ( $\pm$ )-GR24, even if in some

cases this detail is not specified. Since stereochemistry was shown to be an important issue for SLs activity (Scaffidi *et al.*, 2014) this could lead to inconsistent results among independent studies.

The molecular mechanisms underlying the AM hyphal branching are still poorly known. SLs treatment boosts fungal metabolism, leading to increased ATP production and mitochondrial division (Besserer *et al.*, 2006; 2008). Our data from RNA sequencing of germinated spores of *G. margarita* after the GR24 treatment confirmed Besserer and colleague's findings, revealing the up-regulation of the expression of mitochondrial genes (Salvioli *et al.*, 2016). The differentially expressed genes involved in fungal respiration after the treatment are listed in Table 1. In addition, other genes resulted GR24-responsive (up- or down-regulated). Among them, the most biologically relevant were: a vacuolar amino acid transporter 1-like, a chitin deacetylase, a chitin synthase, a Mating-type HMG-box protein MAT1-2, a multidrug transporter *mdr1* and a cytochrome p450 (Table 1). These data suggests that not only the mitochondrion, but also other cell compartments are sensitive to SLs.

Chitin is a crucial cell wall component of AMF and changes its structural organization along the fungal life cycle (Bonfante, 1988). In addition, chitin oligosaccharides act as signaling molecules eliciting calcium spiking, a key component of a symbiotic pathway involved in the initial stages of root colonization (Genre *et al.*, 2013; Sun *et al.*, 2015). The discovery that GR24 treatment led to an increase in the release of chitin oligomers (Genre *et al.*, 2013) by AMF and, subsequently, to an amplification of the calcium spiking response, offered the first experimental evidence of the interaction between the signaling molecules released by the fungal and plant partners (Bonfante and Genre, 2015). The observation that exposure to chitin oligomers increased the expression of a gene involved in SLs biosynthesis (CCD7) in *Lotus japonicus* together with other genes considered symbiotic markers (Giovannetti *et al.*, 2015), suggests a positive reciprocal feedback in the SL-COs communication system (Fig. 1).

Very little is known about the molecular mechanisms of SLs perception and signal transduction in AMF. So far, homologs of the D14 proteins, the SLs receptors characterized in plants (Waters *et al.*, 2017) have not been found within the only available *Rhizophagus irregularis* genome (Tisserant *et al.*, 2013; Lin *et al.*, 2014). SLs perception may rely on a calcium mediated-process since, by using a transactivator of transcription (TAT) peptide, Moscatiello and colleagues (2014) delivered the bioluminescent calcium reporter aequorin inside *G. margarita* germinating spores



and demonstrated that GR24 evokes a rapid and remarkable elevation in intracellular calcium concentration which is dissipated within 3-4 min. Since oscillations of calcium concentration are often read as a fast cell response to environmental stress (Zhivotovsky and Orrenius, 2011), an alternative hypothesis is that SLs are first perceived by the AMF as foreign molecules (xenobiotics).

To have an overview of fungal responses to SLs we compared transcriptomic data upon GR24 treatment from the two AMF *G. margarita* and *R. irregularis*. We performed GO enrichment analyses starting from public RNA-seq data (NCBI accession numbers: PRJDB3195 for *R. irregularis* and PRJNA267628 for *G. margarita*) (Fig. 2). Many up-regulated genes were related to the nucleus cellular component and DNA-related functions. Interestingly, *R. irregularis* revealed similar patterns with nucleus and organelle as the more enriched cell categories.

Lipid metabolism and/or localization were other enriched categories shared by the two fungal symbionts. Irrespectively of the fact that AMF are auxotrophic for lipids (Bravo *et al.*, 2017; Luginbuehl *et al.*, 2017; Jiang *et al.*, 2017; Keymer *et al.*, 2017), lipids are the dominant form of stored carbon in AMF spores (Beilby and Kidby, 1980; Jabaji-Hare, 1988; Gaspar *et al.*, 1994; Bonfante *et al.*, 1994). The mobilization of lipids has possibly a central role during the germination to produce carbohydrates and cellular bioenergetic potential (Lammers *et al.*, 2001; Besserer *et al.*, 2008). In germinating spores, acetyl CoA-derived from lipids breakdown enters the glyoxylate cycle (Lammers *et al.*, 2001) to produce carbohydrates potentially employed in glycogen and chitin synthesis. Taken in the whole, the data suggest that SLs may activate metabolic pathways leading to lipid recycling. This process is probably central not only for hyphal branching, but also for spore germination in both AMF. SLs analogs were indeed shown to stimulate spore germination of *R. irregularis* and *Glomus claroideum* (Besserer *et al.*, 2006). Also our current experiments suggest a significant increase in *G. margarita* germination rate after GR24 treatment (M. Novero, unpublished results).

More recent RNA-seq experiments were performed by Kamel and colleagues (2017) using *R. irregularis* and *Gigaspora rosea* in association with three phylogenetically distant host plants in comparison with non symbiotic germinating spore treated with GR24 or root exudates. They found a core set of secreted proteins (SP) shared by both AMF. Most of these common SPs are small proteins of unknown function that may represent putative host non-specific effector

proteins. The suggestion that SLs may induce the secretion of proteins relevant for the symbiosis already found a confirmation in the findings of Tsuzuki *et al.* (2016). The putative secreted protein 1 (SIS1), highly induced by GR24, was shown to be essential for the correct establishment of the AM symbiosis (Tsuzuki *et al.* 2016).

Taken in the whole, these results suggest that SLs regulate the expression of many fungal secreted proteins whose activity may be operational during both the pre-symbiotic and symbiotic stages, leading to a positive control on host plant colonization.

### **Strigolactones and prokaryotes: a focus on the endobacteria of AMF**

Recent works have discovered an increasing number of cooperative bacterial-fungal associations (Frey-Klett *et al.*, 2011) and revealing an unexpected level of diversity in these interactions (Olsson *et al.*, 2017). Some AMF possess endobacteria inside their cytoplasm, leading to the most intimate interaction so far described between bacteria and fungi. Irrespective of their genetic and functional diversity, fungal-associated bacterial communities constitute a novel type of microbiota, the fungal microbiota (Desirò *et al.*, 2014, Bonfante and Desirò, 2017). The rod shaped endobacterium *Candidatus Glomeribacter gigasporarum* (CaGg) has a crucial role in the pre-symbiotic life stage of *G. margarita*, enhancing its bioenergetic potential in terms of ATP production (Salvioli *et al.*, 2016). Since it is acknowledged that SLs have an impact on the fungal mitochondrial metabolism (Besserer *et al.*, 2006, 2008), we wondered whether they could be perceived by the endobacterium. It has already been demonstrated that low concentrations of GR24 stimulates nodule formation in the legume-rhizobia interaction (López-Ráez *et al.*, 2017 and references therein). In a recent work McAdam *et al.* (2017) showed that SLs promote infection thread formation probably by influencing the bacterial partner.

When *G. margarita* germinated spores were treated with SLs analogs, CaGg showed a strong increase of the expression of *ftsZ*, a bacterial replication marker (Anca *et al.*, 2009) and an increase in the number of bacteria was observed. The boost of fungal metabolism induced by GR24 may provide energy and nutrients for the bacterium to increase its population.

When compared to a cured line lacking CaGg (Lumini *et al.*, 2007), the *G. margarita* line containing endobacteria revealed a higher level of transcripts involved in mitochondrial respiration (Table 2), a higher ATP production and a more intense oxygen consume (Salvioli *et al.*, 2016; Vannini *et al.*, 2016). Interestingly, similar effects were observed after GR24 treatment

(Table 2). We speculate that both the endobacterium and SLs have the fungal mitochondrion as the first target, and that the presence of *CaGg* could make *G. margarita* more efficient in responding to SLs. This is supported by the observation that a *CaGg* peroxiredoxin encoding gene was specifically activated when *G. margarita* spores were treated with GR24 (Salvioli *et al.*, 2016). Interestingly, this bacterial gene, a marker for ROS-scavenger metabolism, was not activated when spores were treated with H<sub>2</sub>O<sub>2</sub>. The bacterial enzyme could be specifically active against the endogenous ROS produced by the fungal respiration that is boosted by the GR24 treatment.

In summary, current results suggest that SLs are perceived not only by the AMF, but also by their endobacteria. It would be interesting to clarify whether these responses are direct or mediated by the fungal host.

### **The impact of strigolactones on non AM fungi**

Since SLs have a wide distribution throughout the plant kingdom (Delaux *et al.*, 2012; 2014) and are components of root exudates it is likely they could be involved in the communication with other organisms beside AMF and parasitic plants (Garcia-Garrido *et al.* 2009). Indeed, SLs were shown to have an important role in the control of other biotic interactions (Marzec 2016; López-Ráez *et al.*, 2017). These types of investigations are of high relevance as they could highlight commonalities or specificities in genes and signals, including those exchanged in the rhizosphere, that mediate plant responses to pathogenic and symbiotic microbes (Hayachi and Parniske, 2014). In plant-microbe interactions, two mode of actions of SLs can be envisaged: a direct effect on the microbial growth or an indirect effect that may arise during the colonization process as a consequence of changes in the host plant metabolism. After the work of Akyiama *et al.* (2005) on AMF, the effects of SLs on the *in vitro* growth of a number of other plant-interacting fungi have been investigated (Steinkellner *et al.*, 2007; Dor *et al.*, 2011; Torres-Vera *et al.*, 2014; Dekker *et al.*, 2017) with sometimes conflicting results possibly related to the different biological systems, experimental conditions, final concentration and type/mixture of SLs stereoisomers.

The application of GR24 into a hole in the medium in front of a colony did not show effect on hyphal branching of *Paxillus involutus*, *Laccaria bicolor*, *Amanita muscaria*, *Cenococcum geophilum* (ectomycorrhizal fungi), *Piriformospora indica* and *Trichoderma* (beneficial fungi), *Rhizoctonia solani*, *Fusarium oxysporum* and *Verticillium dahliae* (soil-borne pathogens) or

226 *Botrytis cinerea* and *Cladosporium* sp. (pathogen of aerial parts) (Steinkellner *et al.*, 2007). With  
227 a similar assay (GR24 solutions added to fibreglass discs in front of the fungal colony) Torres-  
228 Vera *et al.* (2014) did not observe impact on the growth of *B. cinerea*. Application of eip-GR24  
229 also had no effect on growth of the oomycete *Pythium irregulare* (Blake *et al.*, 2016) or  
230 *Fusarium oxysporum* (Foo *et al.*, 2016).

231 On the other hand, the supply of GR24 embedded in the medium where the fungi were inoculated  
232 led to a reduced radial growth of several plant pathogens (*Fusarium oxysporum* f. sp. *melonis*,  
233 *Fusarium solani* f. sp. *mango*, *Sclerotinia sclerotiorum* and *Macrophomina phaseolina*,  
234 *Alternaria alternata*, *Colletotrichum acutatum* and *Botrytis cinerea*). In addition, slightly  
235 increased hyphal branching was observed for *A. alternata*, *F. solani* f. sp. *mango* and *B. cinerea*  
236 (Dor *et al.*, 2011). In a similar assay GR24 reduced the *Sclerotinia sclerotiorum* colony size by  
237 20% (Decker *et al.*, 2017).

238 The last experimental system was also used by Belmondo *et al.* (2017) who confirmed the  
239 sensitivity to GR24 of *B. cinerea*. The reduction in radial growth was indeed exploited in a  
240 bioassay for the screening of *B. cinerea* knock-out mutants less sensitive to GR24. Two mutants  
241 turned out to be less sensitive to GR24; one is defective of a thioredoxin reductase and the second  
242 is lacking a transcription factor belonging to the GATA family. Interestingly, both mutants  
243 display an impaired ROS metabolism. In addition, an oxidizing effect was observed in the  
244 mitochondrial intermembrane space of a *B. cinerea* strain expressing a redox-sensitive GFP2  
245 upon exposure to GR24. It seems therefore that also in this pathogenic system, in analogy to what  
246 has been observed in AMF, ROS and mitochondria are emerging as mediators of SLs actions.

247 A connection between SLs and ROS was also observed during the early stages of host plant  
248 infection by root parasitic plants (Gonzalez-Verdejo *et al.*, 2006).

249 These results may open new experimental and conceptual perspectives to identify genetic  
250 determinants involved in SLs responses in AMF. In an evolutionary perspective it can be  
251 hypothesized that SLs may have been first perceived by fungi as a stress/xenobiotic signal and  
252 were later co-opted for host detection by AMF (Dor *et al.*, 2011; Belmondo *et al.*, 2017).

253 SLs biosynthetic mutants were also analysed to study the role of SLs on the outcome of plant-  
254 pathogen interactions (Marzec, 2016; Fig. 3). The tomato *slccd8* mutants showed hypersensitivity  
255 to *B. cinerea* (Torres-Vera *et al.*, 2014). Very recently, Decker *et al.* (2017) demonstrated that  
256 *ccd7* and *ccd8* mutants of the moss *Physcomitrella patens* (which is not an AM host) are more

susceptible to *S. sclerotiorum*, *F. oxysporum* and *Irpex* sp. This effect seems to be mediated by the interaction of SLs with other defence-related hormones rather than a direct effect of SLs on the fungal growth (Torres-Vera *et al.*, 2014; Decker *et al.*, 2017). However, no difference in disease development was observed between SL-deficient and wild-type pea challenged with *Fusarium oxysporum* or the oomycete *Pythium irregulare* (Blake *et al.*, 2016). Thus, so far a general role of SLs on biotic stress cannot be defined.

### **The AM symbiosis and SLs at a crossroad of root morphogenesis and phosphorus metabolism**

While SLs play an important function in the early pre-contact stage of the AM symbiosis, by contrast, their role when the fungus develops in root tissues is not fully clear. Understanding this issue is hampered by the fact both SLs and the AM symbiosis influence several aspects of root biology in particular the root system architecture, including the formation of lateral roots which are the preferential site of AM colonization (Matthys *et al.*, 2016; Oláh *et al.*, 2005; Mukherjee and Ané, 2011; Fusconi 2014). Moreover, the AM symbiosis has a deep impact on mineral nutrient metabolism in particular that of phosphorus (P; Smith *et al.*, 2011), which in turn influences the production of SLs. It is in fact known that SLs biosynthesis and exudation are highly dependent on nutrient availability, with an increase in particular under phosphate (Pi) limiting conditions (López-Ráez *et al.*, 2008) when the AM symbiosis can provide major benefits to the host plant. However, the supply of GR24 to plants with high Pi status did not restore AM colonization (Balzergue *et al.*, 2011; Breullin *et al.*, 2010). Further evidence that SLs are not required for P regulation of AM comes from the observation that SL-deficient mutant can still regulate AM in response to P (Foo *et al.*, 2013a).

These observations indicate that nutrient availability/status is therefore a stronger driver in the control of AM colonization and further support the occurrence of a complex and finely tuned endogenous regulation of the process. In the last decade, several studies, on the basis of pharmacological (treatment with the molecule of interest) and genetic approaches (analysis of mutant lines), highlighted the involvement of other phytohormones (Pozo *et al.*, 2015); in addition, for some of them evidence of cross-talk with SLs metabolism is also emerging. In the following paragraphs we will present data on how SLs metabolism is modified upon mycorrhization, also providing potential explanations of the mycorrhizal phenotype in SLs

mutants.

It is worth to mention that non-host plants produce mainly non-canonical SLs like carlactone and derivatives (albeit this has been analyzed mostly in *Arabidopsis*, and may not be valid as a general statement for non-host plants; Abe *et al.*, 2014; Seto *et al.*, 2014); these non-canonical SL forms have been reported to be active on AMF (Mori *et al.*, 2016). In addition, SLs treatment does not induce the formation of the symbiosis in non-host roots (Illana *et al.*, 2011). The non AM host status thus does not depend on SLs but is possibly the consequence of the lack of several symbiotic genes (Delaux *et al.*, 2014). In the context of an evo-devo perspective (Bonfante and Genre, 2008), SLs synthesis genes seems to be operational downstream the genes of the CSP (Oldroyd *et al.*, 2013). Interestingly, two transcription factors of the CSP, NSP1 and NSP2, were shown to act as regulators of SLs biosynthesis (Liu *et al.*, 2011). Indeed CSP mutants in pea display reduced SLs levels in roots consistent with the hypothesis that CSP positively regulates SLs biosynthesis (McAdam *et al.*, 2017). In addition, very recent data showed that NSP1, which is induced in colonized cortical cells during later stages of AM colonization (Takeda *et al.*, 2013) also contributes to the transcriptional program associated with arbuscule degeneration (Floss *et al.*, 2017). Connection elements are therefore emerging between SLs and the CSP which may contribute to the control of the AM symbiosis not only in the early but also in the late stages of the colonization process.

### **SLs biosynthesis is regulated during the AM colonization**

SLs biosynthesis and exudation into the rhizosphere are induced under nutrient limiting condition and during the early stage of the AM symbiosis (Yoneyama *et al.*, 2007; Yoneyama *et al.*, 2013; López-Ráez *et al.*, 2015). Then, when the AMF profusely colonizes the root (later stages) a decrease of SLs content was observed in tomato, lettuce, pea, cowpea and cotton roots (Lendzemo *et al.*, 2009; López-Ráez *et al.*, 2011; 2014; Aroca *et al.*, 2013; Fernández-Aparicio *et al.*, 2010). The SLs reduction in mature mycorrhizas has been related to the activation of a control mechanism to limit over-colonization which could be metabolically costly for the host plant (López-Ráez *et al.*, 2015). However, the molecular bases of this mechanism are not known. Depending on the plant species, different expression profiles of *CCD7* and *CCD8*, the key genes involved in SLs biosynthesis (Fig. 3; Al Babili and Bouwmeester, 2015) and, so far, the most investigated, were detected during late stages of mycorrhizal colonization.

The spatio-temporal expression pattern of the *CCD7* and *CCD8* genes was investigated in tomato during the AM symbiosis establishment in the whole root system in a time course experiment and, through the laser microdissection technology, in different cell populations (López-Ráez *et al.*, 2015). Interestingly, in mycorrhizal roots, *SICCD7* was up-regulated compared to non-mycorrhizal roots in all the considered time points and in cortical cells containing arbuscules compared to the cortical cells without arbuscules. By contrast, the expression of *SICCD8* did not change significantly in any condition. In agreement, no change in *CCD8* expression in the later stage of the symbiosis was also reported in petunia (Breullin *et al.*, 2010). A similar *CCD* expression pattern was observed in the model legume *Medicago truncatula* where only the putative homolog of *CCD7* was up-regulated in mature mycorrhizas (Gomez *et al.*, 2010). However, in the other legume *Lotus japonicus* both *CCD7* and *CCD8* were slightly induced with a comparable expression pattern during the pre-symbiotic (4 days post fungus inoculation - dpi) and late stages (28 dpi) (Guether *et al.*, 2009).

Similarly, high-throughput gene expression analysis in rice mycorrhizal root revealed a strong up-regulation of both *CCD7/OsD17* and *CCD8/OsD10* during the late stage of the symbiosis (Güimil *et al.*, 2005; Fiorilli *et al.*, 2015). Interestingly, both *CCD* genes and the two rice MAX1 homologs (Cardoso *et al.*, 2014) were also found to be strongly expressed in the host large lateral roots (LLR) compared to the non-host fine lateral roots (FLR) in the presence of AMF, suggesting that the SLs biosynthesis is locally, and not systemically, induced by the presence of the fungus (Fiorilli *et al.*, 2015). Interestingly, the two root types displayed a different Pi content: the non-host FLR have a higher level of Pi compared to the host LLR. These data suggest that in FLR the increase in Pi level may repress the SLs biosynthesis, contributing to make this tissue recalcitrant to AM fungal colonization. It is worth to note that in rice other genes, annotated as *CCD8*, are up-regulated during AM colonization (Fiorilli *et al.*, 2015). Although they have not been characterized so far, it can be hypothesized that they may be involved in the regulation of SLs metabolism and of the AM symbiosis.

Even if data are fragmentary, there is evidence of a constant *CCD7* gene activation upon mycorrhization. This activation has been related to the involvement of this enzyme also in the production of AM-induced C<sub>13</sub>/C<sub>14</sub> apocarotenoids such as  $\alpha$ -inol glucoside and mycorradicin (Klingner *et al.*, 1995; Walter *et al.*, 2000; Fester *et al.*, 2002; Vogel *et al.*, 2010). By contrast, the expression of *CCD8*, which is known to specifically catalyze the synthesis of carlactone, a

SLs precursor, is often not regulated by the AM symbiosis.

Remarkably, a SLs reduction was described in mature mycorrhizas (Lendzemo *et al.*, 2009; López-Ráez *et al.*, 2011; 2014; Aroca *et al.*, 2013; Fernández-Aparicio *et al.*, 2010) but this is not mirrored by a down-regulation of the *CCD7* and/or *CCD8* SLs biosynthetic genes (López-Ráez *et al.*, 2015). It is worth to note that SLs biosynthesis is regulated by a negative feedback mechanism that controls *CCD7* and *CCD8* expression (Simons *et al.*, 2007; Snowden *et al.*, 2005). In addition, an activation of *CCD7* in mycorrhizal roots could also mirror the increased production of additional compounds rather than SLs. A recent study could provide a different explanation: among the secreted proteins expressed by *R. irregularis* (Kamel *et al.*, 2017) one sequence (RiSP811) has been annotated as a putative  $\alpha/\beta$  hydrolase, the enzymatic activity of SLs receptors described in plants (Hamiaux *et al.*, 2012; Nakamura *et al.*, 2013; de Saint Germain *et al.*, 2016); interestingly, the gene is induced by GR24 exposure and during root colonization. It would be interesting to investigate whether this protein could interact with and hydrolyze SLs and therefore contribute to the degradation of SLs in mycorrhizal roots.

The transport of SLs can be considered a further component of SLs metabolism in roots. The *Petunia hybrida* ABC transporter PLEIOTROPIC DRUG RESISTANCE 1 (PDR1) functions as a cellular SLs exporter (Kretzschmar *et al.*, 2012). *pdr1* mutants have normal level of orobanchol (the most abundant SLs in petunia) in root tissues, but orobanchol exudation is reduced and, as a consequence, the AM colonization is less efficient than in WT plants (Kretzschmar *et al.*, 2012; Borghi *et al.* 2016). *PDR1* is up-regulated during the AM colonization and upon Pi starvation. In accordance with this result, PhPDR1 promoter activity was localized in the root tip and in the subepidermal cells of the lateral roots corresponding to hypodermal passage cells which are described, in some plant species, to be the cortical entry points for AMF hyphae and in regions containing or flanking fully developed arbuscules (Sharda and Koide, 2008; Kretzschmar *et al.*, 2012). Sub-cellular localization experiment revealed that the PDR1 protein co-localizes with *CCD8/DAD1* in the root tip (Sasse *et al.*, 2015). These data suggest that the regulation of SLs transport might have also a guidance function in the already colonized root, through the induction of intraradical hyphal branching (Kretzschmar *et al.*, 2012; Borghi *et al.*, 2016).

Up to date the only characterized SLs transporters have been identified in Solanaceae species: the PDR1 from petunia (Kretzschmar *et al.*, 2012) and its putative orthologue in *Nicotiana tabacum* PDR6 (Xie *et al.*, 2015a). Due to frequent duplication events, the identification of PDR1



homologues in other plant species could be difficult.

### **The AM colonization of SLs-deficient and insensitive mutants**

Pea, rice, petunia and tomato mutants impaired in SLs biosynthesis or export display a reduced level of AM colonization; however, the morphology of intraradical fungal structures is never affected (Gomez-Roldan *et al.*, 2008; Breullin *et al.*, 2010; Vogel *et al.*, 2010; Guthjar *et al.*, 2012; Kohlen *et al.*, 2012; Kretzschmar *et al.*, 2012; Vogel *et al.*, 2010; Yoshida *et al.*, 2012). Supplementation with GR24 restores the colonization rate of *rms1/dad1/ccd8* mutant plants (Gomez-Roldan *et al.*, 2008, Breullin *et al.*, 2010), suggesting that SLs are important but not essential for the AM establishment and that the effect of SLs on AMF is mainly occurring in the rhizosphere, although supplementation with GR24 could also affect root physiology and, indirectly, AM colonization.

Interesting data on the AM symbiosis are coming from the analysis of SLs insensitive plants, that is plants defective in SLs signaling components (Fig. 3). The *d14* rice mutant, lacking the SLs receptor (Fig. 3), shows a slightly higher AM colonization levels compared to wild type, probably due to the higher SLs exudation which results from a feedback mechanism (Yoshida *et al.*, 2012). Surprisingly, the AM phenotype in SLs perception mutants defective of downstream signaling components such as the rice *d3* and pea *rms4* (Fig. 3) is rather severe with several aborted infection attempts and a significant reduction of arbuscules and vesicles formation (Yoshida *et al.*, 2012; Foo *et al.*, 2013a) despite they have a normal or an even increased SLs exudation (Yoshida *et al.*, 2012, Gutjahr *et al.*, 2015). It is worth to note that D3/RMS4 F-Box protein is shared by SLs and karrikins signaling pathway. Karrikins are a class of molecules found in aqueous smoke extracts that can promote seed germination of many species (Flematti *et al.*, 2004). Thus, it has been hypothesized that the impaired AM phenotype might be the consequence of the lack of activation of the karrikin signaling (Water *et al.*, 2017). In line with this hypothesis, Gutjahr and colleagues (2015) demonstrated that the rice mutant defective of the karrikin receptor *D14-like* (homolog of the *KAI2* of Arabidopsis) is unable to establish the mycorrhizal symbiosis, a condition mirrored by a complete absence of hyphopodia formation. This is so far one of the most clear-cut mycorrhizal phenotypes so far reported. In line with a potential involvement in early stages of the interaction, the *d14-l* mutant does not show the transcriptional response to germinating spores exudates observed in the wild-type, suggesting the

fascinating hypothesis that the fungal exudates may contain a candidate ligand molecule crucial for the symbiosis. On the other hand, due to the fact that D14-like genes have been found in the genomes of basal land plants, including non AM hosts, and that most plants are not dependent on karrikin for seed germination it has also been suggested that an endogenous, karrikin-like (unknown) compound, plant ligand may exist (Guthjar *et al.*, 2015; Waters *et al.*, 2017).

#### **SLs / hormones cross-talk during the AM colonization**

Several studies indicate possible cross-talks between SLs and other hormones in the regulation of the AM symbiosis, and this makes the understanding of the *in planta* role SLs even more challenging.

Change in auxin level in roots upon AM colonization as well as higher AM colonization rates upon exogenous auxin treatments have been observed in different plants (review in House *et al.*, 2007, Gutjahr 2014). Although the development of fungal structures were not affected, a decrease of the mycorrhization level was observed in pea and tomato mutants affected in indol acetic acid (IAA) biosynthesis, transport or signaling (Foo *et al.*, 2013a; Hanlon *et al.*, 2010). In the pea IAA deficient mutant (*bushy*) the low percentage of mycorrhization was ascribed to a lower SLs biosynthesis and exudation (Foo *et al.*, 2005; Foo 2013). Indeed, GR24 treatment could partially restore the AM colonization (Foo 2013). The link between SLs and IAA is strengthened by the recent results obtained by Guillotin and colleagues (2017) who showed a lower AM colonization in the tomato RNAi *Sl-IAA27* line, which has a reduced expression level of an Aux/IAA gene involved in auxin signaling and specifically up-regulated during mycorrhization. Interestingly, the reduced mycorrhization could be elevated with GR24. This study also demonstrated the co-regulation of the NSP1 and the SL biosynthesis gene D27 leading to the hypothesis that *Sl-IAA27* positively regulates mycorrhization by controlling SLs biosynthesis.

Likewise, ABA positively regulates AM development and functionality (Herrera Medina *et al.*, 2007). ABA biosynthesis knock-out mutants in tomato (*notabilis*, *sitiens* and *flacca*) display a down-regulation of *LeCCD7* and *LeCCD8* (López-Ráez 2010) which is mirrored by a lower (about 40%) SLs content in root exudates (López-Ráez and Bowmeester 2008; López-Ráez *et al.*, 2010). Possibly due to this reduced SLs level, the *sitiens* mutant displayed a reduced number of arbuscules (López-Ráez and Bowmeester 2008; López-Ráez *et al.*, 2010), although this has not

been directly tested.

ABA positively interacts with SLs probably at the biosynthetic level (López-Ráez *et al.*, 2010). On the other hand, SLs can also influence ABA biosynthesis: ABA content in tomato roots and leaves of the SLs-deficient mutant *SL-ORT1* was significantly lower than that of WT plants (Wu *et al.*, 2017), although the molecular basis of the *ort1* mutation is not known. This data was also confirmed in SLs deficient mutant line *Slccd8* where reduced levels of the defence hormones JA, SA and ABA were found compared with the WT (Torres-Vera *et al.*, 2014). In tomato, Lotus and lettuce plants, a cross-talk between ABA and SLs has been found in mycorrhizal plants under drought and under salinity stress (Aroca *et al.*, 2013; Liu *et al.*, 2015; Ruiz-Lozano *et al.*, 2016; López-Ráez 2016). Since mycorrhizal symbiosis alleviates drought and salinity stresses, SLs-ABA cross-talk may at the basis of the benefit of the AM symbiosis provides to plants under these unfavourable conditions (López-Ráez, 2016).

Gibberellins (GA) have been described as negative regulators of the AM symbiosis. Exogenous application of GA inhibits AM colonization in a dose dependent manner (El Ghachtouli *et al.*, 1996; Yu *et al.*, 2014; Takeda *et al.*, 2015). Accordingly, the GA biosynthesis mutants displayed a higher number of arbuscules and the DELLA proteins, repressors of GA signaling, are essential for their formation (Foo *et al.*, 2013b; Floss *et al.*, 2013, Yu *et al.*, 2014, Martín-Rodríguez *et al.*, 2015). A cross-talk between SLs and GA is emerging: a SLs-dependent interaction between the SLs receptor, D14, and the GA signaling repressor, SLR1 was reported (Nakamura *et al.*, 2013) and, recently, GA signaling was shown to controls the SLs biosynthesis, through a down-regulation of corresponding genes (Ito *et al.*, 2017). Interestingly, in the SLs-deficient mutant (*SL-ORT1*) GA3 content was higher in root than in the WT, while in leaves, the GA level (in particular GA3 e GA9) showed an opposite trend (Wu *et al.*, 2017). However SL-deficient mutant in pea has no change in GA content of shoot (de Saint Germain *et al.*, 2013). These observations open the question whether the defect in the AM colonization may arise from a lack of SLs or an increase of GA or from balanced fine tuning of the two hormones.

The role of cytokinins (CK) in the AM symbiosis is less explored (Foo *et al.*, 2013b). So far, increase CK level in mycorrhizal plants was reported (Allen *et al.*, 1980; Shaul-Keinan *et al.*, 2002). Recently, it has been demonstrated that both shoot- and root-specific alterations of CK

levels play important roles in the relation between CK homeostasis and the growth effect observed in AM plants (Cosme *et al.*, 2016). By contrast, no AM phenotype was detected in the medicago CK-insensitive mutant *cre1* (cytokinin response 1) defective in a cytokinin receptor, suggesting that at least the CRE1-dependent cytokinin signaling is not essential for the AM symbiosis (Foo *et al.*, 2013b). So far, little evidence of interaction between CK and SLs metabolism has emerged. CK might inhibit SLs biosynthesis (Bainbridge *et al.*, 2005) but contrasting results were obtained for CK content in SLs biosynthesis mutants probably due to the different organs and different species considered. In particular, in pea and Arabidopsis SLs-deficient mutants a reduced levels of cytokinin in xylem sap was observed (Beveridge *et al.*, 1994, 1997a,b; Morris *et al.*, 2001; Foo *et al.*, 2007). A decrease content of dihydrozeatin (dhZ) was also detected in leaves of tomato *SL-ORT1* mutant while the root displayed an increase content of CK than WT plants (Wu *et al.*, 2017). No differences of CK content were observed in shoot apices of rice *d* mutants (Arite *et al.*, 2007) and in shoot tissue of pea SLs-deficient mutant (Foo *et al.*, 2007).

Still little explored is the role of brassinosteroids (BR) in the development of the AM symbiosis. Tomato mutants defective in BR biosynthesis showed decreased mycorrhization (Bitterlich *et al.*, 2014). Interestingly, Wang and colleagues (2013) demonstrated that Arabidopsis BES1 (bri1-EMS-suppressor 1), a positive regulator in BR signaling pathway, is a direct target of MAX2, the F-box protein involved in SLs signaling (Fig. 3), and acts as a negative regulator of SLs signaling pathway to promote shoot branching (Wang *et al.*, 2013).

Overall the deregulation of the AM colonization (lower / higher colonization rate) observed in auxin, ABA and GA mutants indicate that these hormones contribute to control AM establishment. For some of them (auxin, ABA and GA) possible cross-talks with SLs are emerging. While a direct role of SLs on the AMF is evident in the rhizosphere, the situation is definitely more complex inside the root tissues. In fact, a mycorrhizal root is a very heterogeneous environment where local and systemic responses occur. In addition, the AM colonization is a very dynamic process with a high arbuscule turnover. Specific spatio-temporal changes in the synthesis, distribution and/or activity of SLs and other hormones are likely to occur and, in the end, mediate the final outcome of the complex network of interactions.

It is also important to underline that there is a distinction between the early stages of the interactions where the fungal metabolism must be activated to favor the contact with the host (active metabolism, release of signaling molecules...) from the late stages where a fine control over fungal proliferation should be set up to guarantee the beneficial mutualistic association. It is tempting to speculate that SLs and the cross-talk with the other phytohormones may contribute to regulate the complex process controlling mycorrhizal formation and arbuscules turn over.

## Conclusions

SLs are signal molecules with an ancient origin in the plant kingdom. Their ancestral function of regulators of developmental processes has accompanied the increasing biological complexity of land plants (Waters et al., 2017). Their versatility is also witnessed by the fact that during the evolution they have been exploited, once released in the rhizosphere, as a vocabulary to communicate with soil organisms even belonging to different kingdoms (i.e. AMF and associated bacteria) beside parasitic plants. The range of plant-interacting organisms that may be targets of SLs action could be even wider. SLs biosynthetic mutants often show higher susceptibility to pathogens, possibly due to an altered homeostasis of other defence hormones; however, this is not a universal response since the outcome of some plant-microbe interactions is not influenced by the lack of SLs (López-Ráez *et al.*, 2017). To better define the involvement of SLs in plant-pathogen interactions, more detailed studies, possibly extended to different pathosystems, are needed. This information will be instrumental for a safe use of natural or synthetic SLs as innovative tools in the field of agro-biotechnology.

In the specific case of the AM symbiosis studies carried out in the last decade showed that SLs act as positive regulators. Although not essential for the establishment of this mutualistic association, SLs are relevant to achieve a full extent of mycorrhization, primarily by boosting the fungal metabolism and, ultimately, its ability to reach and colonize root tissues. The role of SLs *in planta* is, so far, still ambiguous as the perturbation of SLs biosynthesis and signaling was shown to alter the metabolism of other hormones which also contribute to the correct establishment of the AM symbiosis. In addition, SLs seem to operate in the hub which regulates phosphate metabolism as well as root morphogenesis, two processes that, in host plants, are known to be, to some extent, under the control of the AM symbiosis (Smith *et al.*, 2011; Fusconi, 2014). Understanding the biological relevance of each of the components of this complex

network and how they interact will be the challenging task to be pursued in the future.

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## References

**Abe S, Sado A, Tanaka K, *et al.*** 2014. Carlactone is converted to carlactonoic acid by MAX1 in *Arabidopsis* and its methyl ester can directly interact with AtD14 *in vitro*. *Proceedings of the National Academy of Sciences of the United States of America* **111**, 18084-18089.

**Akiyama K, Matsuzaki K, Hayashi H.** 2005. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* **435**, 824-827.

**Akiyama K, Ogasawara S, Hayashi H.** 2010. Structural requirement of strigolactones for hyphal branching in AM fungi. *Plant Cell Physiology* **51**, 1104-1117.

**Al-Babili S, Bouwmeester HJ.** 2015. Strigolactones, a novel carotenoid-derived plant hormone. *Annual Review in Plant Biology* **66**, 161-186.

**Allen MF, Thomas S, Moore Jr, Christensen M.** 1980. Phytohormone changes in *Bouteloua gracilis* infected by vesicular-arbuscular mycorrhizae. I. Cytokinin increases in the host plant. *Canadian Journal of Botany* **58**, 371-374.

**Anca IA, Lumini E, Ghignone S, Salvioli A, Bianciotto V, Bonfante P.** 2009. The *ftsZ* gene of the endocellular bacterium ‘*Candidatus Glomeribacter gigasporarum*’ is preferentially expressed during the symbiotic phases of its host mycorrhizal fungus. *Molecular Plant-Microbe Interactions* **22**, 302-310.

**Arite T, Iwata H, Ohshima K, Maekawa M, Nakajima M, Kojima M, Sakakibara H, Kyojuka J.** 2007. DWARF10, an RMS1/MAX4/DAD1 ortholog, controls lateral bud outgrowth in rice. *The Plant Journal* **51**, 1019-1029.

**Aroca R, Ruiz-Lozano JM, Zamarreño AM, Paz JA, García-Mina JM, Pozo MJ, López-Ráez JA.** 2013. Arbuscular mycorrhizal symbiosis influences strigolactone production under salinity and alleviates salt stress in lettuce plants. *Journal of Plant Physiology* **170**, 47-55.

**Bainbridge K, Sorefan K, Ward S, Leyser O.** 2005. Hormonally controlled expression of the Arabidopsis *MAX4* shoot branching regulator gene. *The Plant Journal* **44**, 569-580.

**Balergue C, Puech-Pagès V, Bécard G, Rochange SF.** 2011. The regulation of arbuscular mycorrhizal symbiosis by phosphate in pea involves early and systemic signalling events. *Journal of Experimental Botany* **62**, 1049-1060.

**Beilby JP, Kidby DK.** 1980. Biochemistry of ungerminated and germinated spores of the vesicular-arbuscular mycorrhizal fungus *Glomus caledonium*: changes in neutral and polar lipids. *Journal of Lipid Research* **21**, 739-750.

**Belmondo S, Marschall R, Tudzynski P, López Ráez JA, Artuso E, Prandi C, Lanfranco L.** 2017. Identification of genes involved in fungal responses to strigolactones using mutants from fungal pathogens. *Current Genetics* **63**, 201-213.

**Besserer A, Becard G, Roux C, Jauneau A, Sejanon-Delmas N.** 2008. GR24, a synthetic analogue of strigolactones, stimulates mitosis and growth of the arbuscular mycorrhizal fungus *Gigaspora rosea* by boosting its energetic metabolism. *Plant Physiology* **148**, 402-413.

**Besserer A, Puech-Pages V, Kiefer P, et al.** 2006. Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria. *PLoS Biology* **4**, 1239-1247.

**Beveridge CA, Murfet IC, Kerhoas L, Sotta B, Miginiac E, Rameau C.** 1997a. The shoot controls zeatin riboside export from pea roots: evidence from the branching mutant *rms4*. *Plant Journal* **11**, 339-345.

**Beveridge CA, Ross JJ, Murfet IC.** 1994. Branching mutant *rms-2* in *Pisum sativum*. *Plant Physiology* **104**, 953-959.

**Beveridge CA, Symons GM, Murfet IC, Ross JJ, Rameau C.** 1997b. The *rms1* mutant of pea has elevated indole-3-acetic acid levels and reduced root-sap zeatin riboside content but increased



branching controlled by graft-transmissible signal(s). *Plant Physiology* **115**, 1251-1258.

**Bitterlich M, Krügel U, Boldt-Burisch K, Franken P, Kühn C.** 2014. The sucrose transporter SISUT2 from tomato interacts with brassinosteroid functioning and affects arbuscular mycorrhiza formation. *The Plant Journal* **78**, 877-889.

**Blake SN, Barry KM, Gill WM, Reid JB, Foo E.** 2016. The role of strigolactones and ethylene in disease caused by *Pythium irregulare*. *Molecular Plant Pathology* **17**, 680-690.

**Bolger AM, Lohse M, Usadel B.** 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*. **30**(15), 2114-2120.

**Bonfante P, Anca IA.** 2009. Plants, mycorrhizal fungi, and bacteria: a network of interactions. *Annual Review of Microbiology* **63**, 363-383.

**Bonfante P, Balestrini R, Mendgen K.** 1994. Storage and secretion processes in the spore of *Gigaspora margarita* Becker and Hall as revealed by high-pressure freezing and freeze substitution. *New Phytologist* **128**, 93-101.

**Bonfante P, Desirò A.** 2017. Who lives in a fungus? The diversity, origins and functions of fungal endobacteria living in Mucoromycota. *The ISME Journal* 1-9.

**Bonfante P, Genre A.** 2008. Plants and arbuscular mycorrhizal fungi: an evolutionary-developmental perspective. *Trends in Plant Science* **13**, 492-498.

**Bonfante P, Genre A.** 2010. Mechanisms underlying beneficial plant-fungus interactions in mycorrhizal symbiosis. *Nature Communications* **1**, 48.

**Bonfante P, Genre A.** 2015. Arbuscular mycorrhizal dialogues: do you speak “plantish” or “fungish”? *Trends in Plant Science* **20**(3), 150-154.

**Bonfante P.** 1988. The role of the cell wall as a signal in mycorrhizal association. In: Scannerini S, Smith D, Bonfante-Fasolo P, Gianinazzi-Pearson V, eds. *Cell to cell signals in plant, animal and microbial symbiosis* (NATO ASI serie, series H, Vol. 17). Berlin, Germany: Springer Verlag, 219-236.

**Borghi L, Liu GW, Emonet A, Kretschmar T, Martinoia E.** 2016. The importance of strigolactone transport regulation for symbiotic signaling and shoot branching. *Planta* **243**, 1351-1360.

**Bravo A, Brands M, Wewer V, Dörmann P, Harrison MJ.** 2017. Arbuscular mycorrhiza-specific enzymes FatM and RAM2 fine-tune lipid biosynthesis to promote development of arbuscular mycorrhiza. *New Phytologist* **214**, 1631-1645.

**Breuillin F, Schramm J, Hajirezaei M, et al.** 2010. Phosphate systemically inhibits development of arbuscular mycorrhiza in *Petunia hybrida* and represses genes involved in mycorrhizal functioning. *The Plant Journal* **64**, 1002-1017.  
by the arbuscular mycorrhizal fungus *Gigaspora margarita*. *New Phytologist* **203**(3), 1012-1020.

**Cardoso C, Zhang Y, Jamil M, et al.** 2014. Natural variation of rice strigolactone biosynthesis is associated with the deletion of two MAX1 orthologs. *Proceedings of the National Academy of Sciences of the United States of America* **6**, 2379-2384.

**Cook CE, Whichard LP, Turner B, Wall ME, Egley GH.** 1966. Germination of witchweed (*Striga lutea* Lour.): isolation and properties of a potent stimulant. *Science* **154**, 1189-1190.

**Corradi N, Brachmann A.** 2017. Fungal mating in the most widespread plant symbionts? *Trends in Plant Science* **22**, 175-183.

**Cosme M, Ramireddy E, Franken P, Schmülling T, Wurst S.** 2016. Shoot- and root-borne cytokinin influences arbuscular mycorrhizal symbiosis. *Mycorrhiza* **26**, 709-720.

**de Saint Germain A, Clave G, Badet-Denisot MA, et al.** 2016. An histidine covalent receptor and butenolide complex mediates strigolactone perception. *Nature Chemical Biology* **12**, 787-794.

**de Saint Germain A, Ligerot Y, Dun EA, Pillot JP, Ross JJ, Beveridge CA, Rameau C.** 2013. Strigolactones stimulate internode elongation independently of gibberellins. *Plant Physiology* **163**(2), 1012-25.

**Decker EL, Alder A, Hunn S, Ferguson J, et al.** 2017. Strigolactone biosynthesis is evolutionarily conserved, regulated by phosphate starvation and contributes to resistance against phytopathogenic fungi in a moss, *Physcomitrella patens*. *New Phytologist* **216**(2), 455-468.

**Delaux PM, Varala K, Edger PP, Coruzzi GM, Pires JC, Ane JM.** 2014. Comparative phylogenomics uncovers the impact of symbiotic associations on host genome evolution. *PLOS Genetics*. 10:e1004487.

**Delaux PM, Xie X, Timme RE, Puech-Pages V, Lecompte E, Dunand C, Delwiche CF, Yoneyama K, Bécard G, Séjalon-Delmas N.** 2012. Origin of strigolactones in the green lineage. *New Phytologist* **195**, 857-871.

**Desirò A, Salvioli A, Ngonkeu EL, Mondo SJ, Epis S, Faccio A, Kaech A, Pawlowska TE, Bonfante P.** 2014. Detection of a novel intracellular microbiome hosted in arbuscular mycorrhizal fungi. *The ISME Journal* **8**, 257-270.

**Dor E, Joel DM, Koltai YKH, Hershenhorn J.** 2011. The synthetic strigolactone GR24 influences the growth pattern of phytopathogenic fungi. *Planta* **234**, 419-427.

**El Ghachtouli N, Martin-Tanguy J, Paynot M, Gianinazzi S.** 1996. First report of the inhibition of arbuscular mycorrhizal infection of *Pisum sativum* by specific and irreversible inhibition of polyamine biosynthesis or by gibberellic acid treatment. *FEBS Letters* **385**, 189-192.

**Fernández-Aparicio M, García-Garrido JM, Ocampo JA, Rubiales D.** (2010). Colonization of field pea roots by arbuscular mycorrhizal fungi reduces *Orobanch*e and *Phelipanche* species seed germination. *Weed Research* **50**, 262-268.

**Fester T, Schmidt D, Lohse S, Walter MH, Giuliano G, Bramley PM, Fraser PD, Hause B, Strack D.** 2002 Stimulation of carotenoid metabolism in arbuscular mycorrhizal roots. *Planta* **216**, 148-54.

**Fiorilli V, Vallino M, Biselli C, Faccio A, Bagnaresi P, Bonfante P.** 2015. Host and non-host roots in rice: cellular and molecular approaches reveal differential responses to arbuscular mycorrhizal fungi. *Frontiers in Plant Science* **6**, 636.

**Flematti GR, Ghisalberti EL, Dixon KW, Trengove RD.** 2004. A compound from smoke that promotes seed germination. *Science* **305**, 977-

**Floss DS, Levy JG, Lévesque-Tremblay V, Pumplin N, Harrison MJ.** 2013. DELLA proteins regulate arbuscule formation in arbuscular mycorrhizal symbiosis. *Proceedings of the National Academy of Sciences of the United States of America* **110**, 5025-5034.

**Foo E, Blake SN, Fisher BJ, Smith JA, Reid JB.** 2016. The role of strigolactones during plant interactions with the pathogenic fungus *Fusarium oxysporum*. *Planta* **243**(6), 1387-1396.

**Foo E, Bullier E, Goussot M, Foucher F, Rameau C, Beveridge CA.** 2005. The branching gene *RAMOSUS1* mediates interactions among two novel signals and auxin in pea. *The Plant Cell* **17**, 464-474.

**Foo E, Morris SE, Parmenter K, Young N, Wang HT, Jones A, Rameau C, Turnbull CGN, Beveridge CA.** 2007. Feedback regulation of xylem cytokinin content is conserved in pea and *Arabidopsis*. *Plant Physiology* **143**, 1418-1428.

**Foo E, Ross JJ, Jones WT, Reid JB.** 2013b. Plant hormones in arbuscular mycorrhizal symbioses: an emerging role for gibberellins. *Annals of Botany* **111**, 769-779.

**Foo E, Yoneyama K, Hugill CJ, Quittenden LJ, Reid JB.** 2013a. Strigolactones and the regulation of pea symbioses in response to nitrate and phosphate deficiency. *Molecular Plant* **6**, 76-87.

**Foo E.** 2013. The interaction between auxin and strigolactones in pea mycorrhizal symbioses. *Journal of Plant Physiology* **170**, 523-528

**Frey-Klett P, Burlinson P, Deveau A, Barret M, Tarkka M, Sarniguet A.** 2011. Bacterial-fungal interactions: hyphens between agricultural, clinical, environmental, and food microbiologists. *Microbiology Molecular Biology Reviews* **75**, 583-609.

**Fusconi A.** 2014. Regulation of root morphogenesis in arbuscular mycorrhizae: what role do fungal exudates, phosphate, sugars and hormones play in lateral root formation? *Annals of Botany* **113**, 19-33.

**Garcia-Garrido JM, Lenzemo V, Castellanos-Morales V, Steinkellner S, Vierheilig H.** 2009. Strigolactones, signals for parasitic plants and arbuscular mycorrhizal fungi. *Mycorrhiza* **19**, 449-459.

**Gaspar ML, Pollero RJ, Cabello MN.** 1994. Triacylglycerol consumption during spore germination of vesicular-arbuscular mycorrhizal fungi. *Journal of American Oil Chemists' Society*. **71**, 449-452.

**Genre A, Chabaud M, Balzergue C, et al.** 2013. Short-chain chitin oligomers from arbuscular mycorrhizal fungi trigger nuclear  $\text{Ca}^{2+}$  spiking in *Medicago truncatula* roots and their production is enhanced by strigolactone. *New Phytologist* **198**, 190-202.

**Genre A, Russo G.** 2016. Does a Common Pathway Transduce Symbiotic Signals in Plant–

Microbe Interactions? *Frontiers in Plant Science* **7**, 96.

**Giovannetti M, Mari A, Novero M, Bonfante P.** 2015. Early *Lotus japonicus* root transcriptomic responses to symbiotic and pathogenic fungal exudates. *Frontiers in Plant Science* **6**, 480.

**Gomez SK, Javot H, Deewatthanawong P, Torres-Jerez I, Tang Y, Blancaflor EB, Udvardi MK, Harrison MJ.** 2009. *Medicago truncatula* and *Glomus intraradices* gene expression in cortical cells harboring arbuscules in the arbuscular mycorrhizal symbiosis. *BMC Plant Biology* doi.org/10.1186/1471-2229-9-10.

**Gomez-Roldàn V, Fermas S, Brewer PB, et al.** 2008. Strigolactone inhibition of shoot branching. *Nature* **455**, 189-94.

**González-Verdejo CI, Barandiaran X, Moreno MT, Cubero JI, Di Pietro A.** 2005. A peroxidase gene expressed during early developmental stages of the parasitic plant *Orobancha ramosa*. *Journal of Experimental Botany* **57**, 185-192.

**Guether M, Balestrini R, Hannah MA, Udvardi MK, Bonfante P.** 2009. Genome-wide reprogramming of regulatory networks, transport, cell wall and membrane biogenesis during arbuscular mycorrhizal symbiosis in *Lotus japonicus*. *New Phytologist*. **182**, 200-212.

**Guillotin B, Etemadi M, Audran C, Bouzayen M, Bécard G, Combier JP.** Sl-IAA27 regulates strigolactone biosynthesis and mycorrhization in tomato (var. MicroTom). 2017. *New Phytologist* **213**, 1124-1132.

**Güimil S, Chang HS, Zhu T, et al.** 2005. Comparative transcriptomics of rice reveals an ancient pattern of response to microbial colonization. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 8066-8070.

**Gutjahr C, Gobbato E, Choi J, et al.** 2015. Rice perception of symbiotic arbuscular

mycorrhizal fungi requires the karrikin receptor complex. *Science* **350**, 1521-1524.

**Gutjahr C, Parniske M.** 2013. Cell and developmental biology of arbuscular mycorrhiza symbiosis. *Annual Review of Cellular and Developmental Biology* **29**, 593-617.

**Gutjahr C, Radovanovic D, Geoffroy J, *et al.*** 2012. The half-size ABC transporters STR1 and STR2 are indispensable for mycorrhizal arbuscule formation in rice. *The Plant Journal*. **69**, 906-920.

**Gutjahr C.** 2014. Phytohormone signaling in arbuscular mycorrhiza development. *Current Opinion in Plant Biology* **20**, 26-34.

**Hamiaux C, Drummond RS, Janssen BJ, Ledger SE, Cooney JM, Newcomb RD, Snowden KC.** 2012. DAD2 is an  $\alpha/\beta$  hydrolase likely to be involved in the perception of the plant branching hormone strigolactone. *Current Biology* **22**, 2032-2036.

**Hanlon MT, Coenen C.** 2011. Genetic evidence for auxin involvement in arbuscular mycorrhizal initiation. *New Phytologist* **189**, 701-709.

**Hayachi M, Parniske M.** 2014. Symbiosis and pathogenesis: what determines the difference? *Current Opinion in Plant Biology* **20**.

**Herrera-Medina MJ, Steinkellner S, Vierheilig H, Bote JAO, Garrido JMG.** 2007. Absciscic acid determines arbuscule development and functionality in the tomato arbuscular mycorrhiza. *New Phytologist* **175**, 554-564.

**House B, Mrosk C, Isayenkov S, Strack D.** 2007. Jasmonates in arbuscular mycorrhizal interactions. *Phytochemistry*, **68**, 101-110.

**Illana A, García-Garrido JM, Sampedro I, Ocampo JA, Vierheilig H.** 2011. Strigolactones seem not to be involved in the non susceptibility of arbuscular mycorrhizal (AM) non host plants to AM fungi. *Botany* **89**, 285-288.

**Ito S, Yamagami D, Umehara M, *et al.*** 2017. Regulation of strigolactone biosynthesis by gibberellin signaling. *Plant Physiology* **174**(2), 1250-1259.

**Jabaji-Hare S.** 1988. Lipid and fatty acid profiles of some vesicular-arbuscular mycorrhizal fungi: contribution to taxonomy. *Mycologia* **80**, 622-629.

**Jiang Y, Wang W, Xie Q, *et al.*** 2017. Plants transfer lipids to sustain colonization by mutualistic mycorrhizal and parasitic fungi. *Science* **356**(6343):1172-1175.

**Kamel L, Tang N, Malbreil M, San Clemente H, Le Marquer M, Roux C, Frei dit Frey N.** 2017. The comparison of expressed candidate secreted proteins from two arbuscular mycorrhizal fungi unravels common and specific molecular tools to invade different host plants. *Frontiers in Plant Science* **8**, 124 doi: 10.3389/fpls.2017.00124

**Kapulnik Y, Koltai H.** 2014. Strigolactone involvement in root development, response to abiotic stress, and interactions with the biotic soil environment. *Plant Physiology* **166**, 561-569.

**Keymer A, Pimprakar P, Wewer V, *et al.*** 2017. Lipid transfer from plants to arbuscular mycorrhiza fungi. *eLife* **6**, e29107.

**Klingner A, Bothe H, Wray V, Marner FJ.** 1995. Identification of a yellow pigment formed in maize roots upon mycorrhizal colonization. *Phytochemistry* **38**, 53-55.

**Kohlen W, Charnikhova T, Lammers M, *et al.*** 2012. The tomato CAROTENOID CLEAVAGE DIOXYGENASE8 (SICCD8) regulates rhizosphere signaling, plant architecture and affects reproductive development through strigolactone biosynthesis. *New Phytologist* **196**, 535-547.

**Koltai H.** 2011. Strigolactones are regulators of root development. *New Phytologist* **190**(3), 545-549.



**Kretzschmar T, Kohlen W, Sasse J, et al.** 2012. A petunia ABC protein controls strigolactone-dependent symbiotic signalling and branching. *Nature* **483**, 341-44.

**Lammers P, Jun J, Abubaker J, et al.** 2001. The glyoxylate cycle in an arbuscular mycorrhizal fungus. Carbon flux and gene expression. *Plant Physiology* **127**, 1287-1298.

**Langmead B, Salzberg SL.** 2012. Fast gapped-read alignment with Bowtie 2. *Nature Methods* **9**(4), 357-359.

**Lendzemo V, Kuyper TW, Vierheilig H.** 2009. Striga seed-germination activity of root exudates and compounds present in stems of Striga host and nonhost (trap crop) plants is reduced due to root colonization by arbuscular mycorrhizal fungi. *Mycorrhiza* **19**, 287-294.

**Lin K, Limpens E, Zhang Z, et al.** 2014. Single nucleus genome sequencing reveals high similarity among nuclei of an endomycorrhizal fungus. *PLoS Genetics* **10**(1): e1004078.

**Liu J, He H, Vitali M, et al.** 2015. Osmotic stress represses strigolactone biosynthesis in *Lotus japonicus* roots: exploring the interaction between strigolactones and ABA under abiotic stress. *Planta* **241**, 1435-1451.

**Liu W, Kohlen W, Lillo A, et al.** 2011. Strigolactone biosynthesis in *Medicago truncatula* and rice requires the symbiotic GRAS-type transcription factors NSP1 and NSP2. *The Plant Cell* **23**, 3853-3865.

**López-Ráez JA, Shirasu K, Foo E.** 2017. Strigolactones in plant onteractions with beneficial and detrimental organisms: the Yin and Yang. *Trends in Plant Science* **22**, 527-537.

**López-Ráez JA, Bouwmeester HJ.** 2008. Fine-tuning regulation of strigolactone biosynthesis under phosphate starvation. *Plant Signaling and Behavior* **3**, 963-965.

**López-Ráez JA, Charnikhova T Gòmez-Roldàn V, et al.** 2008. Tomato strigolactones are

derived from carotenoids and their biosynthesis is promoted by phosphate starvation. *New Phytologist* **178**, 863-874.

**López-Ráez JA, Charnikhova T, Fernández I, Bouwmeester H, Pozo MJ.** 2011. Arbuscular mycorrhizal symbiosis decreases strigolactone production in tomato. *Journal of Plant Physiology* **168**, 294-297.

**López-Ráez JA, Fernández I, García JM, Berrio E, Bonfante P, Walter MH, Pozo MJ.** 2015. Differential spatio-temporal expression of carotenoid cleavage dioxygenases regulates apocarotenoid fluxes during AM symbiosis. *Plant Science*. **230**, 59-69.

**López-Ráez JA, Kohlen W, Charnikhova T, et al.** 2010. Does abscisic acid affect strigolactone biosynthesis? *New Phytologist* **187**, 343-354.

**Love MI, Huber W, Anders S.** 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* **15**(12), 550.

**Luginbuehl LH, Menard GN, Kurup S, Van Erp H, Radhakrishnan GV, Breakspear A, Oldroyd GED, Eastmond PJ.** 2017. Fatty acids in arbuscular mycorrhizal fungi are synthesized by the host plant. *Science* **356**(6343), 1175-1178.

**Lumini E, Bianciotto V, Jargeat P, Novero M, Salvioli A, Faccio A, Bécard G, Bonfante P.** 2007. Presymbiotic growth and spore morphology are affected in the arbuscular mycorrhizal fungus *Gigaspora margarita* cured of its endobacteria. *Cellular Microbiology* **9**, 1716-1729.

**Martín-Rodríguez JA, Molinero-Rosales N, Tarkowska D, Ruíz-Rivero O, García-Garrido JM.** 2015. Role of gibberellins during arbuscular mycorrhizal formation in tomato: new insights revealed by endogenous quantification and genetic analysis of their metabolism in mycorrhizal roots. *Physiologia Plantarum* **154**(1), 66-81.

**Marzec M.** 2016. Perception and signaling of strigolactones. *Frontiers in Plant Science* **7**, 1260

doi:10.3389/fpls.2016.01260

**Matthys C, Walton A, Struk S, Stes E, Boyer FD, Gevaert K, Goormachtig S.** 2016. The Whats, the Wheres and the Hows of strigolactone action in the roots. *Planta* **243**, 1327-1337.

**McAdam EL, Hugill C, Fort S, Samian E, Cottaz S, Davies NW, Reid JB, Foo E.** 2017. Determining the site of action of strigolactones during nodulation. *Plant Physiology* DOI: <https://doi.org/10.1104/pp.17.00741>

**Mori N, Nishiuma K, Sugiyama T, Hayashi H, Akiyama K.** 2016. Carlactone-type strigolactones and their synthetic analogues as inducers of hyphal branching in arbuscular mycorrhizal fungi. *Phytochemistry* **130**, 90-98.

**Morris SE, Turnbull CGN, Murfet IC, Beveridge CA.** 2001. Mutational analysis of branching in pea. Evidence that *Rms1* and *Rms5* regulate the same novel signal. *Plant Physiology* **126**, 1205-1213.

**Moscatiello R, Sello S, Novero M, Negro A, Bonfante P, Navazio L.** 2014. The intracellular delivery of TAT-aequorin reveals calcium mediated sensing of environmental and symbiotic signals by the arbuscular mycorrhizal fungus *Gigaspora margarita*. *New Phytologist* **203**(3), 1012-1020.

**Mukherjee A, Ané JM.** 2011. Germinating spore exudates from arbuscular mycorrhizal fungi: molecular and developmental responses in plants and their regulation by ethylene. *Molecular Plant-Microbe Interactions* **24**, 260-270.

**Nakamura H, Xue YL, Miyakawa T, et al.** 2013. Molecular mechanism of strigolactone perception by DWARF14. *Nature Communication* **4** 2613 10.1038/ncomms3613

**Oláh B, Brière C, Bécard G, Dénarié J, Gough C.** 2005. Nod factors and a diffusible factor from arbuscular mycorrhizal fungi stimulate lateral root formation in *Medicago truncatula* via the

DMI1/DMI2 signalling pathway. *The Plant Journal* **44**, 195-207.

**Oldroyd GED.** 2013. Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. *Nature Reviews Microbiology* **11**, 252-263.

**Olsson S, Bonfante P, Pawlowska TE,** 2017. Ecology and evolution of fungal-bacterial interactions. In: Dighton J, Oudem P (eds). *The Fungal Community: Its Organization and Role in the Ecosystem*, CRC Press Taylor & Francis, Boca Raton, FL, USA, pp.563-583.

**Pozo MJ, López-Ráez JA, Azcón-Aguilar C, García-Garrido JM.** 2015. Phytohormones as integrators of environmental signals in the regulation of mycorrhizal symbioses. *New Phytologist* **205**, 1431-1436.

**Ruiz-Lozano JM, Aroca R, Zamarreno AM, Molina S, Andreo-Jimenez B, Porcel R, Garcia-Mina JM, Ruyter-Spira C, Lopez-Raez JA.** 2016. Arbuscular mycorrhizal symbiosis induces strigolactone biosynthesis under drought and improves drought tolerance in lettuce and tomato. *Plant, Cell and Environment* **39**, 441-452.

**Salvioli A, Ghignone S, Novero M, Navazio L, Venice F, Bagnaresi P, Bonfante P.** 2016. Symbiosis with an endobacterium increases the fitness of a mycorrhizal fungus, raising its bioenergetics potential. *ISME Journal* **10**, 130-144.

**Sasse J, Simon S, Gubeli C, Liu GW, Cheng X, Friml J, Bouwmeester H, Martinoia E, Borghi L.** 2015. Asymmetric localizations of the ABC transporter PaPDR1 trace paths of directional strigolactone transport. *Current Biology* **25**, 647-655.

**Scaffidi A, Waters MT, Sun YK, Skelton BW, Dixon KW, Ghisalberti EL, Flematti GR, Smith SM.** 2014. Strigolactone hormones and their stereoisomers signal through two related receptor proteins to induce different physiological responses in *Arabidopsis*. *Plant Physiology* **165**, 1221-1232.

**Seto Y, Sado A, Asami K, Hanada A, Umehara M, Akiyama K, Yamaguchi S.** 2014. Carlactone is an endogenous biosynthetic precursor for strigolactones. *Proceedings of the National Academy of Sciences of the United States of America* **111**, 1640-1645.

**Sharda JN, Koide RT.** 2008. Can hypodermal passage cell distribution limit root penetration by mycorrhizal fungi? *New Phytologist* **180**, 696-701.

**Shaul-Keinan O, Gadkar V, Ginzberg I, et al.** 2002. Hormone concentrations in tobacco roots change during arbuscular mycorrhizal colonization with *Glomus intraradices*. *New Phytologist* **154**, 501-507.

**Simons JL, Napoli CA, Janssen BJ, Plummer KM, Snowden KC.** 2007. Analysis of the DECREASED APICAL DOMINANCE genes of petunia in the control of axillary branching. *Plant Physiology* **143**, 697-706.

**Smit P, Raedts J, Portyanko V, Debellé F, Gough C, Bisseling T, Geurts R.** 2005. NSP1 of the GRAS protein family is essential for rhizobial Nod factor-induced transcription. *Science* **308**, 1789-1791.

**Smith SE, Iver Jakobsen I, Grønlund M, Smith FA.** 2011. Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. *Plant Physiology* **156**, 1050-1057.

**Snowden KC, Simkin AJ, Janssen BJ, Templeton KR, Loucas HM, et al.** 2005. The Decreased apical dominance1/ *Petunia hybrida* CAROTENOID CLEAVAGE DIOXYGENASE8 gene affects branch production and plays a role in leaf senescence, root growth, and flower development. *The Plant Cell* **17**, 746-759.

**Spatafora JW, Chang Y, Benny GL, et al.** 2016. A phylum-level phylogenetic classification of zygomycete fungi based on genomescale data. *Mycologia* **108**, 1028-1046.

**Steinkellner S, Lendzemo V, Langer I, Khaosad T, Schweiger P, Toussaint JP, Vierheilig H.** 2007. Flavonoids and strigolactone in root exudates as signals in symbiotic and pathogenic plant fungus interactions. *Molecules* **12**, 1290-1306.

**Sun H, Tao J, Gu P, Xu G, Zhang Y.** 2016. The role of strigolactones in root development. *Plant Signaling and Behavior* **11**, 1, e1110662.

**Sun J, Miller JB, Granqvist E, Wiley-Kalil A, Gobbato E, Maillet F, Cottaz S, Samain E, Venkateshwaran M, Fort S, *et al.*** 2015. Activation of symbiosis signaling by arbuscular mycorrhizal fungi in legumes and rice. *The Plant Cell* **27**(3), 823-38.

**Takeda N, Handa Y, Tsuzuki S, Kojima M, Sakakibara H, Kawaguchi M.** 2015. Gibberellin regulates infection and colonization of host roots by arbuscular mycorrhizal fungi. *Plant Signaling and Behaviour* **10**(6), e1028706.

**Tisserant E, Malbreil M, Kuo A, *et al.*** 2013. Genome of an arbuscular mycorrhizal fungus provides insight into the oldest plant symbiosis. *Proc Natl Acad Sci USA* **110**, 20117-20122.

**Torres-Vera R, García JM, Pozo MJ, López-Ráez JA.** 2014. Do strigolactones contribute to plant defence? *Molecular Plant Pathology* **15**(2), 211-216.

**Tsuzuki S, Handa Y, Takeda, N, Kawaguchi M.** 2016. Strigolactone-induced putative secreted protein 1 is required for the establishment by the arbuscular mycorrhizal fungus *Rhizophagus irregularis*. *Molecular Plant-Microbe Interactions* **29**(4), 277-286.

**Vannini C, Carpentieri A, Salvioli A, *et al.*** 2016. An interdomain network: The endobacterium of a mycorrhizal fungus promotes antioxidative responses in both fungal and plant hosts. *New Phytologist* **211**, 265-275.

**Vogel JT, Walter MH, Giavalisco P, *et al.*** 2010. SLCCD7 controls strigolactone biosynthesis,

shoot branching and mycorrhiza-induced apocarotenoid formation in tomato. *The Plant Journal* **61**, 300-311.

**Walter MH, Fester T, Strack D.** 2000. Arbuscular mycorrhizal fungi induce the non-mevalonate methylerythritol phosphate pathway of isoprenoid biosynthesis correlated with accumulation of the 'yellow pigment' and other apocarotenoids. *The Plant Journal* **21**, 571-578.

**Wang Y, Sun S, Zhu W, Jia K, Yang H, Wang X.** 2013. Strigolactone/MAX2-induced degradation of brassinosteroid transcriptional effector BES1 regulates shoot branching. *Developmental Cell* **27**, 681-688.

**Waters MT, Gutjahr C, Bennett T, Nelson DC.** 2017. Strigolactone signaling and evolution. *Annual Review of Plant Biology* **68**, 291-322.

**Wu Y, Dor E, Hershenhorn J.** 2017. Strigolactones affect tomato hormone profile and somatic embryogenesis. *Planta* **245**, 583-594.

**Xie X, Wang G, Yang L, Cheng T, Gao J, Wu Y, Xia Q.** 2015. Cloning and characterization of a novel *Nicotiana tabacum* ABC transporter involved in shoot branching. *Physiologia Plantarum* **153**, 299-306.

**Yoneyama K, Yoneyama K, Takeuchi Y, Sekimoto H.** 2007. Phosphorus deficiency in red clover promotes exudation of orobanchol, the signal for mycorrhizal symbionts and germination stimulant for root parasites. *Planta* **225**, 1031-1038.

**Yoshida S, Kameoka H, Tempo M, et al.** 2012. The D3 F-box protein is a key component in host strigolactone responses essential for arbuscular mycorrhizal symbiosis. *New Phytologist* **196**, 1208-1216.

**Yu N, Luo D, Zhang X, et al.** 2014. A DELLA protein complex controls the arbuscular mycorrhizal symbiosis in plants. *Cell Research* **24**, 130-133.

**Zhivotovsky B, Orrenius S.** 2011. Calcium and cell death mechanisms: a perspective from the cell death community. *Cell Calcium* **50**(3), 211-21.

**Zipfel C, Oldroyd GED.** 2017. Plant signalling in symbiosis and immunity. *Nature* **543**, 328-336.



**Table 1.** Differentially expressed genes in *G. margarita* germinating spores after 1 week GR24 treatment. A fold change cutoff of +/- 0.5 and an FDR of < 0.05 have been used (Salvioli *et al.*, 2016).

Transcript ID	Log2 Fold Change	Sequence description
<i>Genes involved in fungal respiration</i>		
comp35750_c0	1.3	cytochrome c oxidase subunit 1
comp15252_c0	0.65	ubiquinol-cytochrome c reductase complex core protein 2 precursor
comp15565_c0	0.83	nadh dehydrogenase Fe-S protein 5
comp18263_c0	0.39	nadh dehydrogenase 1 alpha subcomplex 6
comp31224_c0	0.7	ubiquinol-cytochrome c reductase complex 17 kd protein
comp32142_c0	2.25	nadh dehydrogenase subunit 4l
comp34943_c1	1.26	nadh dehydrogenase subunit 52037
comp36626_c0	0.48	cytochrome c oxidase subunit va
comp36884_c0	0.7	cytochrome c oxidase assembly protein cox-16
comp37253_c0	1.17	cytochrome c
comp6965_c0	0.6	ubiquinol-cytochrome c reductase complex 14 kDa protein
comp7520_c0	0.78	nadh dehydrogenase
<i>Genes involved in other pathways</i>		
comp37189_c0	1.18	vacuolar amino acid transporter 1-like
comp37057_c0	1.07	chitin deacetylase
comp5264_c0	-1.65	chitin synthase
comp38121_c0	-0.85	mating type protein mat1-2-1
comp9271_c0	-4.18	ABC multidrug transporter mdr1
comp39141_c0	1.9	cytochrome P450

**Table 2.** Differentially expressed genes in *G. margarita* germinating spores containing (B+) or not (B-) the endobacteria and after GR24 treatment. A fold change cutoff of +/- 0.5 and an FDR of < 0.05 have been used (Salvioli *et al.*, 2016).

**B+ vs B-**

Transcript ID	Log2 Fold Change	Sequence description
comp35650_c2	0.88	cytochrome c oxidase subunit 1
comp34209_c0	0.54	nadh dehydrogenase subunit1
comp33766_c0	0.25	nadh-ubiquinone oxidoreductase
comp29917_c0	3	nadh dehydrogenase

**B+ GR24 vs B- GR24**

Transcript ID	Log2 Fold Change	Sequence description
comp35750_c0	1.65	apocytochrome b
comp32142_c0	1.44	nadh dehydrogenase subunit 4l
comp34871_c0	1.39	cytochrome c oxidase subunit 3
comp35009_c0	1.36	mitochondrial protein, putative
comp34943_c1	1.28	nadh dehydrogenase subunit 5
comp35650_c2	1.12	cytochrome c oxidase subunit 1

## Figure legends

**Figure 1.** The scheme illustrates the potential interactions between the signaling molecules released by the fungal and plant partners in the AM symbiosis. SLs treatment leads to an increase in the release of chitin oligomers by AMF and, as a consequence, to an amplification of the calcium spiking response in the host plant (Genre *et al.*, 2013); COs induce the expression of CCD7, a SLs biosynthetic gene (Giovannetti *et al.*, 2015), although it has not been proved that this leads to induced SLs production. SLs treatment also stimulates the release of fungal secreted protein, such as SIS1 that positively regulates the AM colonization (Tsuzuki *et al.* 2016).

**Figure 2.** List of the enriched GO (Gene Ontology) categories in germinating spores of *R. irregularis* (A) and *G. margarita* (B) after 1 week GR24 treatment. The differential expression analysis was performed as described in Salvioli *et al.* (2016). Briefly, raw reads libraries were trimmed with Trimmomatic V.0.36 (Bolger *et al.*, 2014) and aligned on the reference transcriptomes (Lin *et al.*, 2014; Salvioli *et al.*, 2016) using bowtie2 (Langmead and Salzberg 2012). The DESeq2 1.12.4 Bioconductor package (Love *et al.*, 2014) was used for the identification of differentially expressed genes. Gene Ontology (GO) enrichments were performed with the AgriGO web platform (<http://bioinfo.cau.edu.cn/agriGO/>) and plotted with ggplot2 R package.

**Figure 3.** Biosynthesis and signaling pathway of SLs.

CCD: CAROTENOID CLEAVAGE DIOXYGENASE;

D: DWARF (*Oryza sativa* genes);

DAD: DECREASED APICAL DOMINANCE (*Petunia hybrida* genes);

MAX: MORE AUXILLARY GROWTH (*Arabidopsis thaliana* genes);

RMS: RAMOSUS (*Pisum sativum* genes).

**Figure 4.** Effect of SLs on the host plant, the AM fungus and in its endobacteria during the establishment of AM symbiosis.